PB87-100707

Growth Inhibition and Morphological Effects Several Chemicals in ´Arabidopsis thaliana (L.)´Heynh

Corvallis Environmental Research Lab., OR

1986

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TECHNICAL REPORT DATA (Please read Instructions on the reverse before complet)		
1. REPORT NO. EPA/600/J-86/129	2.	3. PB87-100707
4. TITLE AND SUBTITLE Growth Inhibition and Morphological Effects Several Chemicals in Arabidopsis thaliana (L.)Heynh (Journal Version)		5. REPORT DATE 1986 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Hilman C. Ratsch and Debra Johndro		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS US EPA		10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory Office of Research and Development U.S.Environmental Protection Agency Corvallis, OR 97333		13. TYPE OF REPORT AND PERIOD COVERED Journal article 14. SPONSORING AGENCY CODE EPA 600/02
15. SUPPLEMENTARY NOTES 1986. Environmental Toxicology and Chemistry 5:55-60, January.		

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

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U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
INFORMATION SERVICE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to Public	Unclassified	7
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA Form 2220-1 (9-73)

EPA/600/J-86/129
JOURNAL ARTICLE

Environmental Toxicology and Chemistry Vol. 5, pp. 55-60, 1986
Printed in the USA. Pergamon Press Ltd.

0730-7268/86 $3.00 + .00
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Environmental Toxicology

GROWTH INHIBITION AND MORPHOLOGICAL EFFECTS OF SEVERAL CHEMICALS IN *ARABIDOPSIS THALIANA* (L.) HEYNH.

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(*Received* 27 *August* 1984; *Accepted* 6 *March* 1985)

Abstract—A plant life-cycle bioassay using *Arabidopsis thaliana* (L.) Heynh. was used to evaluate effects of several toxic chemicals. Plants were grown in vermiculite in a hydroponic system, with test chemicals added to the nutrient solution reservoir. Reduction in total biomass followed different patterns with the chemicals tested, and sensitivity increased in the order: fly ash < 2-(2-ethoxyethoxy)ethanol (DGME) < dalapon < trichloroacetic acid (TCA) < copper sulfate < bromacil < metribuzin. DGME, TCA and dalapon induced unusual morphological effects in which flower development was altered and pollination prevented. Reduced biomass, altered distribution of plant dry weight among vegetative, reproductive and mature seed, and morphological effects indicate that a plant life-cycle test is a valuable screening test.

Keywords—*Arabidopsis* Bromacil Copper sulfate Dalapon 2-(2-Ethoxyethoxy)ethanol (DGME) Fly ash Metribuzin Trichloroacetic acid (TCA)

INTRODUCTION

The U.S. Environmental Protection Agency (EPA) is developing a series of phytotoxic tests to screen chemicals for potential effects on terrestrial environments. Most are acute, short-term toxicity tests dealing with a specific stage of plant development. In this report, we evaluate a plant life-cycle bioassay developed to detect morphological changes as well as acute and chronic phytotoxicity caused by test chemicals.

The test species is *Arabidopsis thaliana* (L.) Heynh., a small, herbaceous winter annual in the Cruciferae family. It is widely distributed throughout Europe, Asia, Japan and North American. However, it is best known as a laboratory organism used in research on developmental genetics, growth and developmental physiology, metabolism and mutation. The characteristics that make it suitable for a life-cycle test include its small size, short life cycle, large seed production and the absence of major culturing problems.

Effects on the *Arabidopsis* life cycle were evaluated by testing several chemicals using a modified hydroponic culture and treatment method.

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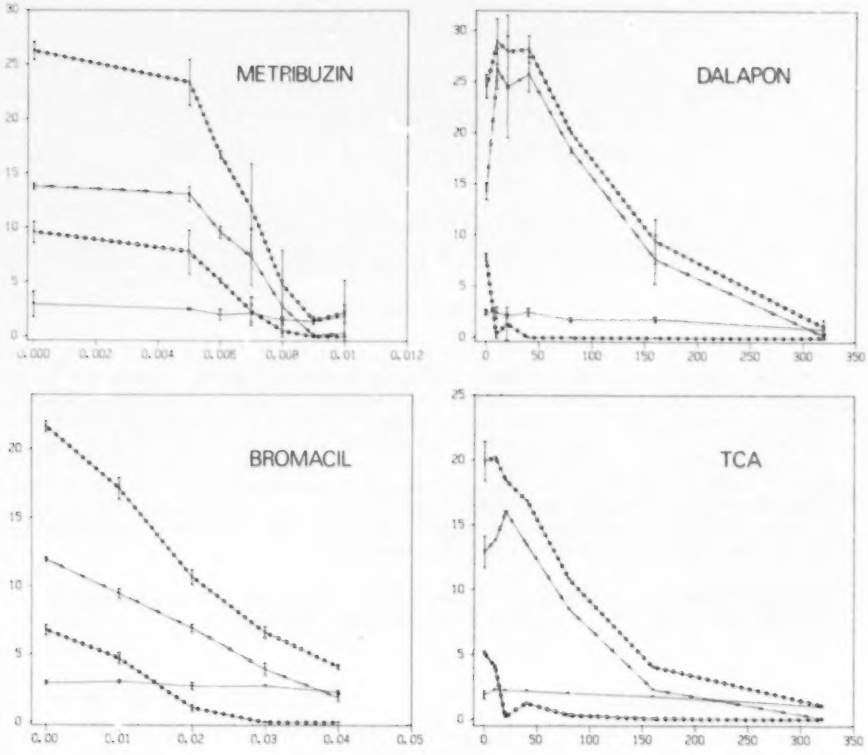
MATERIALS AND METHODS

Arabidopsis thaliana (L.) Heynh. (Columbia wild-type) seeds were originally obtained in 1980 from Dr. G.P. Redei (University of Missouri). The seeds used in this study were progeny from that lot. They were stored in sealed glass vials at room temperature and used for a period of from 2 months to 3 years after harvest.

The plants were grown using a modified double-pot hydroponic system [1] in which an 8-in polypropylene pot was filled with horticultural-grade vermiculite (No. 2 grind topped with 0.5 cm No. 3 grind). This was nested above a 7-in pot that served as a reservoir for the nutrient solution [2]. Nutrients and water moved from the reservoir to the vermiculite via polyester wicks draped into the solution. Seeds were placed on the vermiculite by suspending them in a dilute agar solution, which dripped onto the surface through a separatory funnel [3], or by placing a template with 73 evenly spaced holes on the surface and inserting a single seed into each hole. The latter method yielded uniformly distributed plants and was adopted for testing. The vermiculite was premoistened and pots were placed in a mist chamber for the first 7 d. Germination was routinely greater than 95%.



Experiments were done in a greenhouse with supplemental (sodium vapor HID (high intensity discharge) lamps) lighting to ensure at least a 16-h photoperiod. Light levels varied greatly from winter to summer, and thus the length of the life cycle and the total biomass at harvest varied seasonally. Chemical effects were compared with controls grown at the same time and all effects are expressed in relation to those controls.

Technical-grade herbicides were obtained from either manufacturers or chemical supply companies: bromacil (5-bromo-3-sec-butyl-6-methyluracil), copper sulfate ($CuSO_4 \cdot 5H_2O$), dalapon (2,2-dichloropropionic acid), carbitol R or DGME (2-(2-ethoxyethoxy)ethanol), metribuzin (4-amino-6-tert-butyl-3-(methylthio)-as-triazin-5(4H)-one), TCA (trichloroacetic acid, sodium salt) and a smelter fly ash. The fly ash was obtained from a Tacoma copper smelter baghouse sample collected by EPA regional staff. The major constituents were identified as Pb (18.2% by weight), As (13.1%), Cu (7.9%), Fe (3.1%), Sb (2.7%) and Zn (2.4%), with minor constituents (Bi, Sn, Cd, As, Se, Ni and Cr) ranging from 12 to 7,430 μg/g. Each chemical, except fly ash, is water-soluble, relatively nonvolatile, stable in aqueous solution and phytotoxic, and all have a low acute oral toxicity in animals. There is also information available about the effects of these chemicals on plant biomass and their modes of toxic action. Stock solutions of each chemical were prepared in distilled water by weighing or pipetting enough material to obtain a concentration of 1.0 g/L (1.0 ml/L in the case of DGME). Test solutions were made by adding the appropriate amount of stock to the nutrient solution. Test chemicals were added to the reservoirs when the plants had 7 to 10 rosette leaves. Three replicate pots were used for each chemical concentration except TCA. The fly ash was mixed with No. 2 vermiculite on a weight/weight basis in a large rotary mixer. This was covered with 0.5 cm of the fine-grind vermiculite. Exposure in this test started when the roots penetrated into the contaminated material (about 3 d). After 10 d, a plastic screen (2 × 3 mm mesh) was placed around the plants to prevent cross-contam-

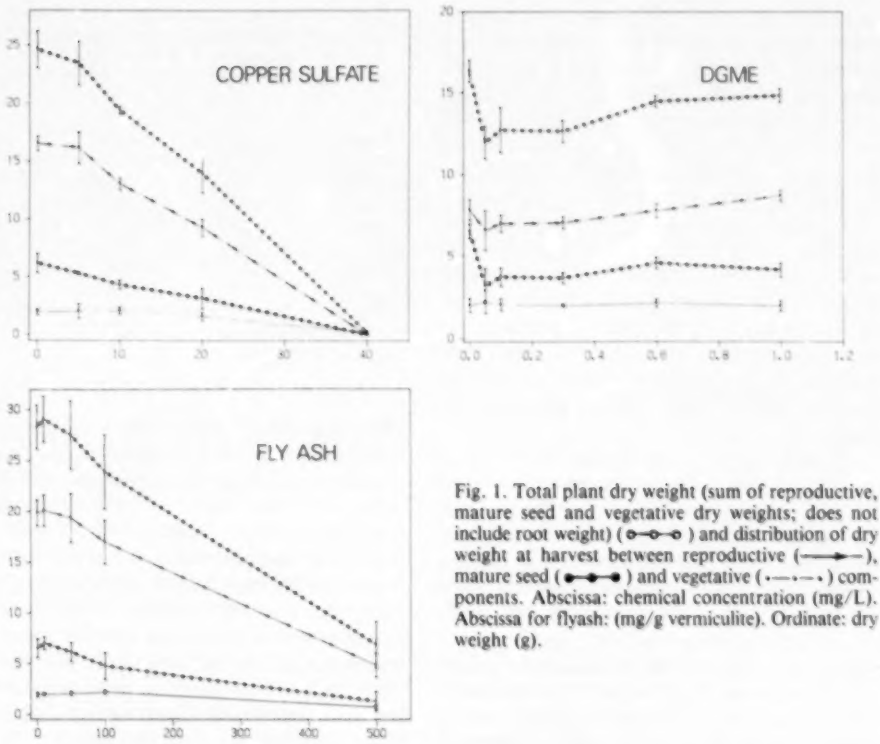


Fig. 1. Total plant dry weight (sum of reproductive, mature seed and vegetative dry weights; does not include root weight) (o—o—o) and distribution of dry weight at harvest between reproductive (——►——), mature seed (●—●—●) and vegetative (·—·—·) components. Abscissa: chemical concentration (mg/L). Abscissa for flyash: (mg/g vermiculite). Ordinate: dry weight (g).

ination and catch any seed that dropped from the plants.

Plants were harvested when all the plots reached maturity (mature seed). Seeds were collected by removing the screen, placing the pot horizontal and shaking the stems over a large piece of paper. Seeds were separated from debris by passing them through screens; they were air-dried at room temperature for 48 h before weighing.

Reproductive biomass consisted of all plant parts above the rosette leaves, including racemes, cauline leaves, flower parts and immature siliques, but excluding mature seed. The main stems were cut 2 cm above the rosette leaves; the tissues were collected and oven-dried at 70°C for 48 h and then weighed. Vegetative biomass included all remaining aerial tissues (primarily the rosette leaves). Vegetative tissue was collected from each plot, then ovendried at 70°C for 48 h and weighed. Total biomass was determined by adding the weights for all components (excluding roots).

Fifty percent reduction (X_{50}) was estimated using a linear regression of total dry weight versus concentration for points on the linear portion of the curve (control weights not included in the regression) and using the formula

$$X_{50} = \frac{1/2\,y - a}{b}$$

The approximate standard error of the estimate was computed using an estimate of experimental error by pooling the control variation and the variation around the regression line.

RESULTS

Total biomass (grams dry weight) is shown for each test chemical concentration in Figure 1. Values for the controls (zero concentration) were similar between tests but varied from a low of 15.4 to a high of 30.7 g/pot. The variations are correlated with the time of year in which the experiments were conducted; maximum dry weight at harvest occurred during summer months and minimum values during the winter. Chemical sensitivity, as determined by the concentration that

suppressed growth by 50%, is shown in Table 1. Effect threshold concentrations are not well defined for most chemicals, but for metribuzin the value appears to be approximately 0.005 mg/L and, for dalapon, approximately 50 mg/L. DGME had very little effect at the concentrations shown; however, at 10 ml/L (not shown), total biomass was less than 10% of controls.

The relative distribution of dry weight between vegetative, reproductive and mature seed components is also shown in Figure 1. In mature control plants, 7 to 13% of the total dry weight (excluding roots) was vegetative tissue, primarily the rosette leaves; 25 to 37% was mature seeds; and 51 to 66% consisted of other reproductive structures (racemes, cauline leaves, siliques and immature seeds).

The partitioning of dry weight in the treated plants varied with increasing concentrations of test chemical. The vegetative weight was the least sensitive component, probably because the plants were treated when the rosette leaves were partially mature. Nevertheless, significant supression was noted for copper sulfate, metribuzin and the fly ash (treatment continuous from seedling stage). Mature seed weight was the most sensitive component. Effects were noted at the lowest concentration and seed weight showed the largest percent change. The reproductive dry weight component (excluding mature seeds) was generally affected at somewhat higher concentrations than was seed weight.

Some chemicals caused different patterns of dry weight partitioning. Some caused a uniform decrease in dry weight among all components (copper sulfate and fly ash). Others caused an increase in one component and a decrease in another. This was most dramatically shown in the lower-concentration treatments of dalapon and TCA. In those cases, there was no apparent effect on vegetative tissue, but the reproductive tissue weights increased while the numbers and weights of mature seeds decreased sharply. This interesting observation allowed detection of a specific result that would have been overlooked had total biomass production been the only screening criteria. Bromacil and metrizubin reduced mature seed weight at a concentration lower than that which affected reproductive weight; these concentrations had little effect on vegetative weight.

The response to DGME was peculiar in two respects. Even at low concentrations, seed weights were decreased by about 30% whereas the other tissues were unaffected or only slightly reduced. At

Table 1. Nominal concentration (X_{50}) of chemical required to reduce total biomass and mature seed (grams dry weight) by 50%

Chemical	Total X_{50} (mg/L)	SE (X_{50})	Seeds[a] X_{50} (mg/L)
Metribuzin	0.007	0.0016	0.007
Bromacil	0.022	0.0004	0.015
$CuSO_4$	21.7	1.27	19
TCA	100	9.1	12
Dalapon	141	12.8	4
Fly ash	335[b]	43.6	290[b]

[a]Graphical interpolation.
[b]mg/g of vermiculite.

increasing concentrations, seed weights were still significantly lower than the controls, but the other tissues associated with reproduction were stimulated. Also, there was a peculiar morphological effect that was not evident in biomass results. At the higher concentrations, no mature siliques formed on the main axis of the racemes. Careful observation revealed that as the flowers opened the ovaries elongated unnaturally until the stigma protruded outside the enclosed petals. The four stamens elongated normally, resulting in the anthers remaining below the stigma surface throughout flower development. Thus the mature pollen did not contact the stigma and pollination was prevented (Fig. 2). The siliques formed from abnormal flowers failed to elongate and seed was not formed.

Normally, all six anthers remain below the stigma surface throughout flower bud development and four stamens elongate as the flower opens, pushing their anthers above the stigma surface where they release the mature pollen. The pollen is sticky and not easily dispersed into the air, which accounts for the absence of cross-pollination even when the plants are crowded together. Once fertilized, the central ovary elongates to form the silique, which is normally 12 to 16 mm long [4,5]. Siliques containing only unfertilized ovules failed to elongate and remained 3.0 to 3.5 mm long.

Similar inhibition of pollination was observed in plants treated with dalapon and TCA. At concentrations of 5 and 10 mg/L, pollination was prevented in the same manner as described above, but as development continued additional effects were observed. Flower buds near the terminus of the main axis remained joined at the tips of the bud, preventing the flower from opening normally. The ovary continued to elongate as previously de-

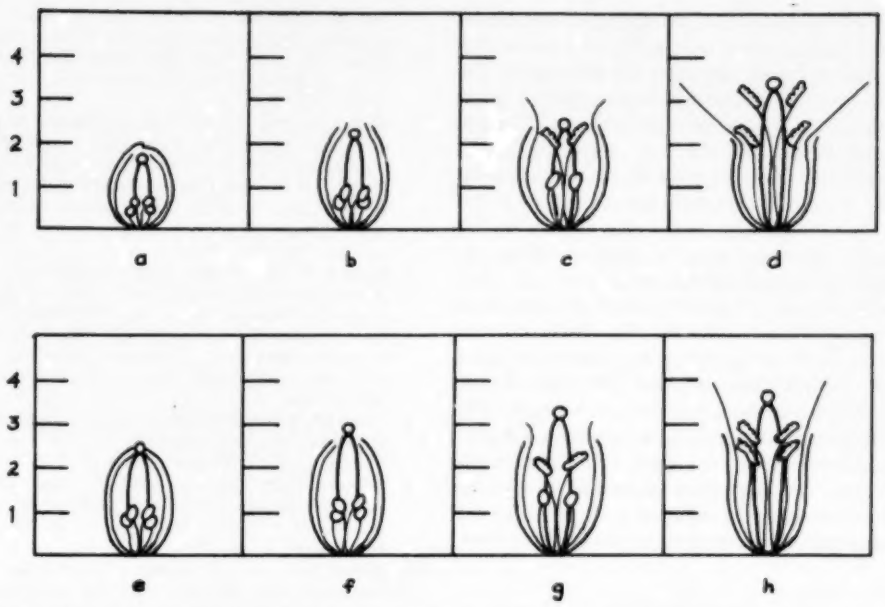


Fig. 2. The early stages in *Arabidopsis thaliana* flower development arranged in developmental sequence for control plants (a–d) and DGME-treated plants (e–h): closed flower bud (**a**); elongating ovary with stigma beginning to protrude from the bud (**b**); partially open flower with immature anthers (**c**); fully open flower with mature anthers at the same level or above stigma (**d**); elongated closed bud with stigma beginning to protrude (**e**); ovary elongated beyond petal length (**f**); partially open flower with ovary protruding above the flower (**g**); open flower with mature anthers below the stigma surface (**h**). The diagrammatic representation of flower parts are those used by Muller [4].

scribed, but folded over within the closed petals. The buds took on a globular shape or occasionally the bud was forced open, releasing a folded 3 to 5 mm silique.

DISCUSSION

Anthropogenic chemicals can cause a toxic reaction in plants in numerous ways. Some act by inhibiting reactions involved in photosynthesis, some inhibit respiration, some are enzyme inhibitors, some are plant growth regulators and many act in ways not yet determined. Phytotoxicity tests to screen for unknown effects caused by new or untested chemicals should include as many different types of responses as reasonably possible. The tests currently proposed by EPA and the Organization for Economic Cooperation and Development include tests of seed germination, root elongation, genetic mutation and others. This work on the phytotoxicity of various chemicals in *Arabidopsis* is intended to pave the way for implementing a plant life-cycle test that will be useful in identifying responses not evident in other tests. *Arabidopsis* is already used extensively as a test organism. Redei [6] proposed a mutagenicity test based on *Arabidopsis*. Others have used it to describe salinity effects [7], herbicide inhibition of root development [8], heavy metal effects [9] and morphological effects [10], to name but a few.

The value of this *Arabidopsis* bioassay lies in its ability to identify chemical effects on essential processes in the plant's life cycle. The change in the way growth is partitioned between vegetative, reproductive and mature seed components is important because the reduction in one component relative to the others and the concentration at which this occurs are critical to assessing the effect of a chemical on plant development. For example, the chemical concentration required to reduce seed weight may be several times lower than that required to reduce total dry weight. It is important to know that the same chemical concentration may cause little or no effect on vegetative growth while causing a severe effect on seed production.

Some of the chemicals used in these tests were chosen to allow comparisons with other work.

Garten and Frank [11] determined the threshold effect concentration on top biomass production for radish, barley, soybeans and bush beans. The lowest nominal concentrations required to produce significant effects for bromacil, dalapon, TCA and copper sulfate were 0.1, 0.1, 10 and 100 mg/L, respectively. The responses of *Arabidopsis* to the same chemicals were more sensitive (Table 1) and gave a different ordering of relative toxicity. The largest difference was for dalapon, unless the response to seed production is used. In other work, we have studied the effect of bromacil on lettuce seedling development. No significant effect was evident at concentrations below 64 mg/L (unpublished data), whereas 50% reduction in *Arabidopsis* biomass occurred at 0.02 mg/L. This difference is not surprising, since bromacil inhibits photosynthesis and obviously is not particularly phytotoxic to other plant functions. These differences dramatize the utility of a phytotoxicity test that reveals effects on all of the life-cycle processes.

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